Supplementary Materials

An integrative analysis of the InR/PI3K/Akt network identifies the dynamic response to Insulin signaling

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Content

Supplementary Tables

Table S1 (related to Figure 2): Datasets corresponding to InsulinNet-PPI.

Bait proteins: List of 20 canonical components used as bait proteins.
Raw data: PPIs from TAP-MS data without filtering non-specific interactions.
Canonical interactions: List of canonical interactions identified in unfiltered network and the corresponding SAINT scores.

InsulinNet-PPI: List of high confidence PPIs (InsulinNet-PPI) identified with SAINT score cutoff >= 0.95.

Literature overlap: List of InsulinNet-PPIs supported by other literature evidences and/or predicted interactions (see Experimental Procedures)

Relevant overlap: List of overlapping PPIs between InsulinNet-PPI and other relevant networks such as MAPK (Friedman et al., 2011), DPiM (Guruharsha et al., 2011) and Glatter et al (Glatter et al., 2011). These networks were complied from the supplementary materials of the corresponding publications.

Enrichment: Enrichment analysis of overlap between InsulinNet-PPI with other relevant networks. To compute the expected overlap, we generated 1000 randomized network for the common baits with same number of prey proteins as real network. Prey proteins are randomly selected from genes expressed in S2R+ cells. Overlap between randomized InsulinNet-PPI is compared to relevant network to estimate the expected overlap.
PRS: Data used to generate positive reference set. Note only PPIs with 3 or more evidences are selected for the analysis.
RRS: Data used to generate random reference set. Note RRS is randomly sampled from this list.

Table S2 (Related to Figure 2): Datasets corresponding to the prey proteins identified in InsulinNet-PPI.

Unique preys: List of 554 unique prey proteins that are part of InsulinNet-PPI. Note that this list includes baits proteins that are also identified as prey proteins.

GO enrichment: Results from GO enrichment analysis of the prey proteins. Enriched GO Biological Process category was listed along with the negative log of the p-values.

Ortholog mapping: List of human orthologs of the InsulinNet-PPI prey proteins. Orthologs were identified using DIOPT tool.

Disease enrichment: List of enriched disease category within the InulinNet-PPI prey proteins. Enrichment analysis was performed using DIOPT-DIST dataset.

Gene expression: Gene expression values for prey proteins in S2R+ cell line and other relevant cell lines. RNA-Seq data with corresponding FPKM values are compiled from modENCODE (http://www.modencode.org/).

Table S3 (Related to Figure 3): Datasets corresponding to InsulinNet-RNAi

Amplicons: List of 480 genes selected for RNAi screen and its corresponding RNAi reagents

Raw data: RNAi screening results. Fold changes and amplicons information for 480 screened genes under each condition tested (pERK_0, pERK_10, pERK_30, pAkt_0 pAkt_10, pAkt_30).

Hits: List of genes selected as hits (regulates pAKT, pERK or both) based on the RNAi screens (InsulinNet-RNAi).

GO enrichment: Enriched GO Biological Process category for the hits and corresponding negative log of the p-values.

Overlap with other RNAi screens: Results from comparative analysis of InsulinNet-RNAi with other relevant RNAi screens.

MS overlap Enrichment: Data from comparative analysis of TAP-MS/MS dynamics with RNAi screes. Data corresponds to figure 3E.

Table S4 (Related to Figure 4): Datasets corresponding to InsulinNet-Phospho

Raw data: List of phosphosites identified using TMT-labeling approach. Data includes quantification of Phosphosites at each time point (baseline, 10 min and 30 min) in duplicates.

Fold changes values are computed by taking average of duplicates and comparing it with baseline condition.

Dynamic sites: List of dynamic sites identified (InsulinNet-Phospho) and the corresponding dynamic classification.

GO enrichment: Enriched biological process GO terms proteins with dynamic phosphosites.

Enriched motifs: List of enriched consensus sequence motif identified using MotifX program. Motif enrichment was computed for each dynamic class separately.

KS network: Predicted kinase-substrate network using NetPhorest program. For each dynamic phosphosite, Netphorest identifies the corresponding upstream kinase using linear motif atlas.

Overlap: Overlapping InuslinNet-Phospho proteins with InsulinNet-PPI and InsulinNet-RNAi.

Table S5 (Related to Figure5): Datasets corresponding to InsulinNet (integrated InsulinNet-PPI, InsulinNet-RNAi and InsulinNet-Phopsho datasets).

Table S6 (Related to Figure 6): Datasets corresponding to COMPLEAT and SignedPPI analysis.

Stable: List of proteins complexes identified as significantly enriched in all three-time points (Baseline, 10 min and 30 min). COMPLEAT complex ID, score and p-values corresponding all three-time points are shown.

Dynamic: List of proteins complexes identified as dynamic complexes (see Experimental Procedures).

Literature: Subset of enriched complexes that are annotated as literature-curated complexes at COMPLEAT. This data corresponds to figure 6D.

Complex Sign: Inferred activation/inhibition relationship between the core-pathway and associated protein complexes in InsulinNet. Signs are predicted using SignPredictor tool (see Experimental Procedures).

Supplementary Figures

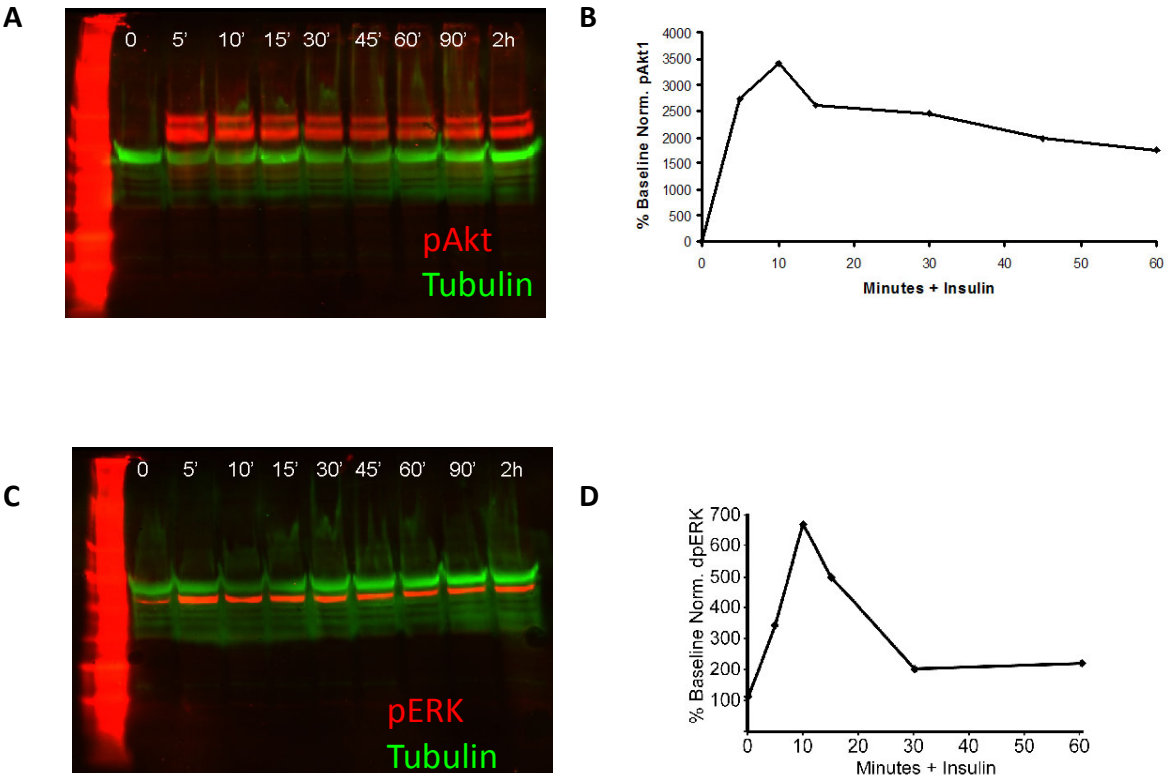


Figure S1 (Related to Figure 1): Time course of Insulin stimulation assessed by levels of phospho-Akt1 (ser 505) and dually phosphorylated ERK. (**A** and **B**) There is no detectable pAkt1 in S2R+ cells at baseline, and levels of pAkt1 peak at 10 minutes after induction with Insulin. pAkt1 levels drop (but stay higher than baseline) at later time points. (**C** and **D**) Levels of pERK also peak between 5-10 minutes after induction with Insulin and drop to near baseline levels at later time points

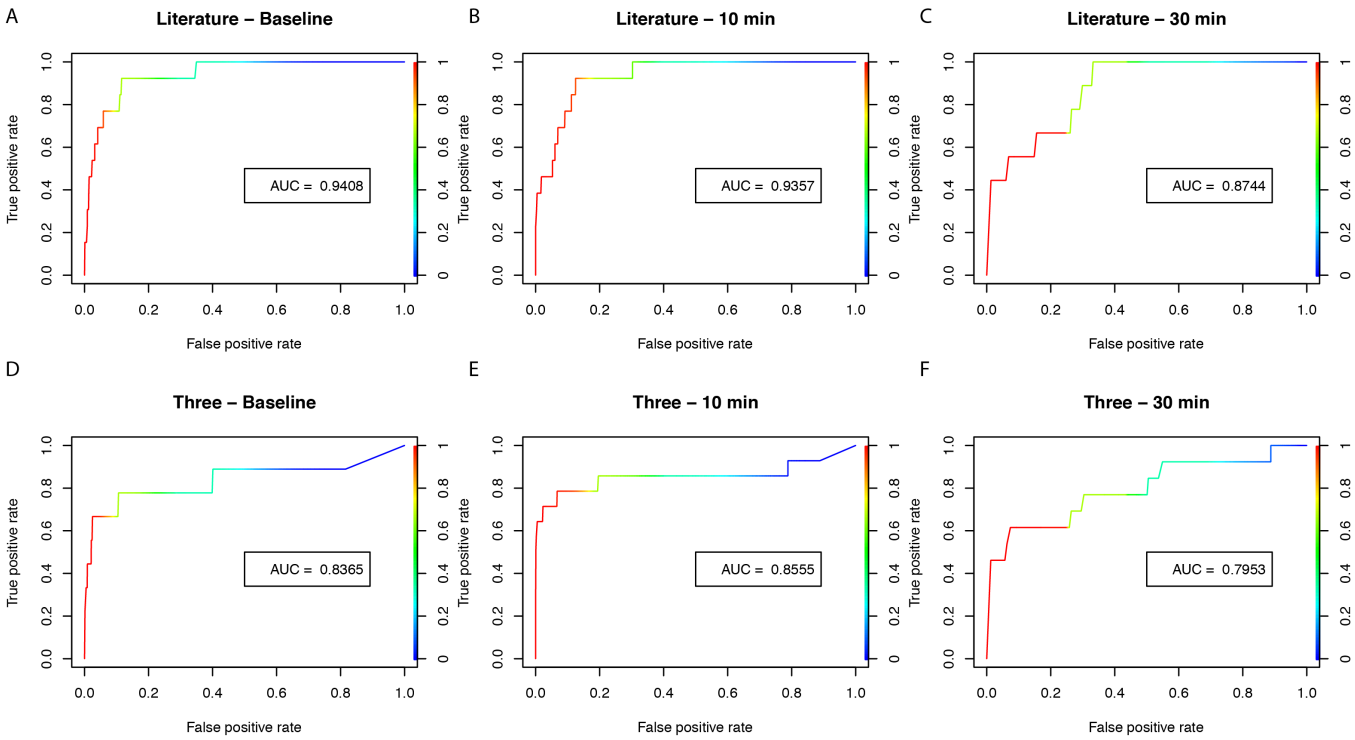
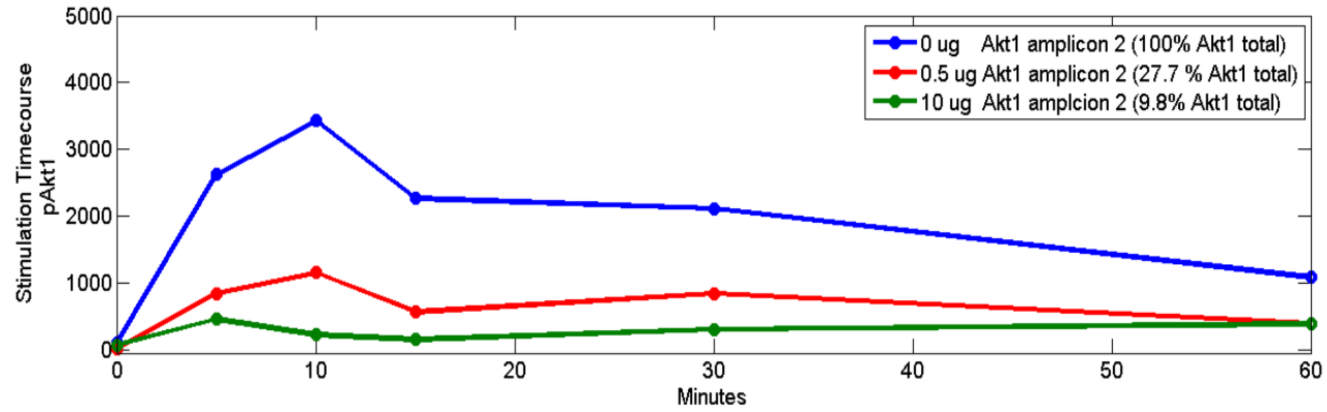


Figure S2 (Related to Figure 2): ROC plot showing the performance of SAINT score based on PRS and RRS. (A) Assessment of baseline network using literature curated PRS and corresponding size controlled RRS. (B) and (C) corresponds to 10 min and 30 min network respectively, PRS and RRS are same as (A). (D-F) Same as (A-C) but the PRS is based on previously published PPIs (including the ones from high-throughput screens) that are supported by 3 or more evidences (see Experimental Procedures).

A



B

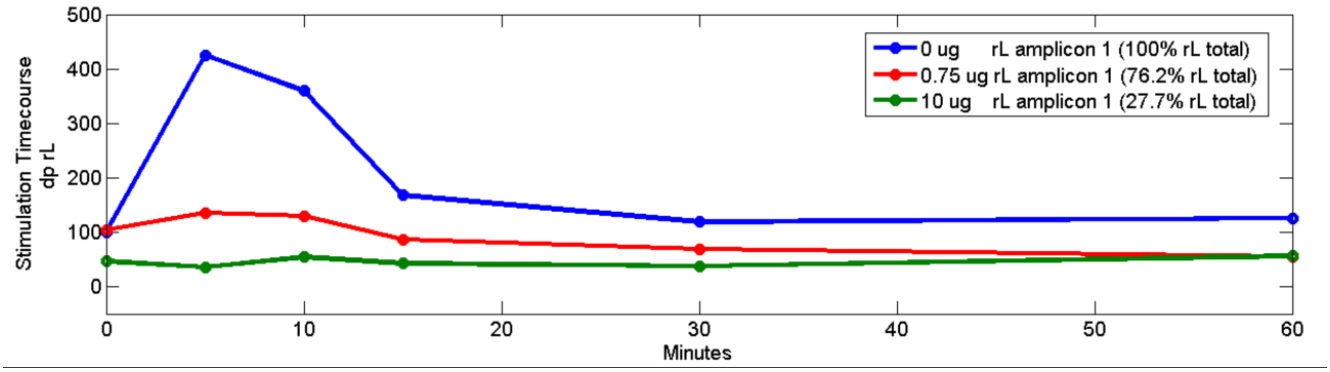


Figure S3 (Related to Figure 3): Plot showing the effect of knocking down of Akt and ERK in the functional RNAi screen. (**A**) RNAi knockdown of Akt reduces the levels of Insulin-induced pAkt. (**B**) RNAi knockdown of ERK reduces the levels of Insulin-induced pERK

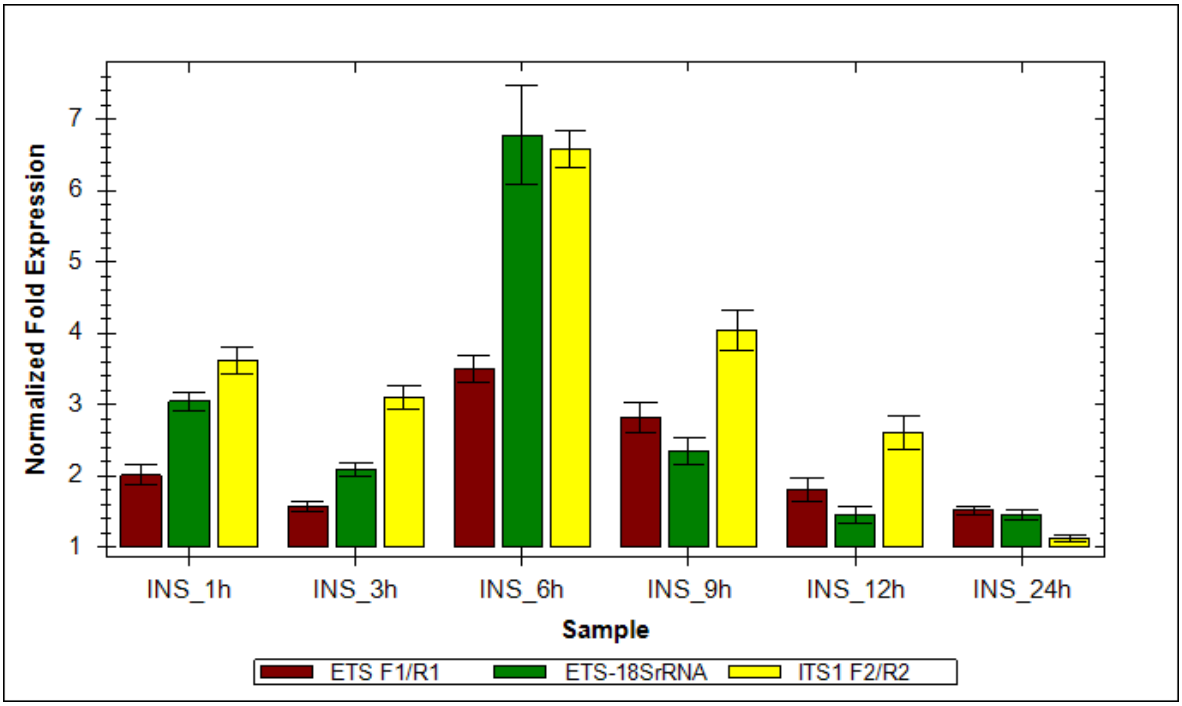


Figure S4 (Related to Figure 7): Characterizing the role of insulin signaling in ribosome biogenesis. Fold-change calculated by normalizing the expression value by baseline condition.

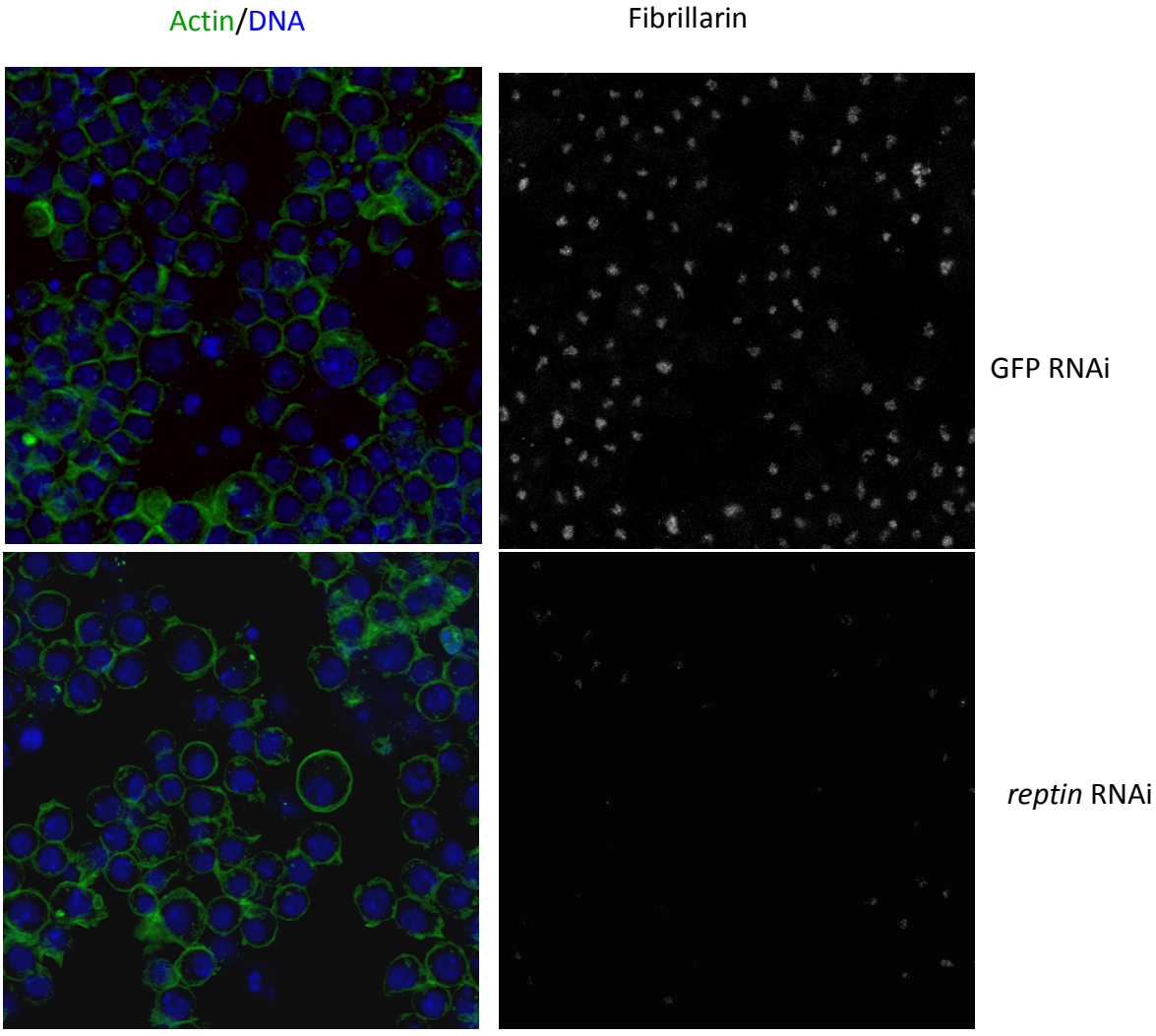


Figure S5 (Related to Figure 7): Charactering the role of reptin in nucleolar morphology in S2R+ cells. Similar but weaker effects are seen with pontin RNAi in S2R+ cells (data not shown). See also Demontis and Perrimon (2009).